Lycopene and Citrulline Contents in Watermelon (*Citrullus lanatus*) Fruit with Different Ploidy and Changes during Fruit Development

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Abstract

Lycopene and citrulline contents of 9 diploids (2X) and their artificially induced autotetraploids (4X) and autotriploids (3X) were determined in mature fruit. Changes in lycopene and citrulline during fruit development was studied using fruit of variety XYXA with three different ploidy levels from 15 to 40 days after pollination (DAP). Polyploids, especially triploids, had higher levels of lycopene than their diploid progenitors, and triploids tended to contain more lycopene than tetraploids. While levels of citrulline were low and more variable, levels tended to be higher in polyploids than diploids, with triploids tending to be higher than tetraploids. There were only trace amounts of lycopene in fruit of watermelon assayed at 15 DAP, but levels increased rapidly, reaching a maximum value at 37 DAP in tetraploid fruit and 34 DAP in triploid and diploid fruit. The maximum lycopene value of diploid fruit was lower than that of either polyploid fruit. The Lycopene content decreased slightly in over-mature fruit. The maximum citrulline value occurred in fruit that was near maturity. The citrulline content peak was earliest in 2X fruit and was slightly later in 3X and 4X fruit. Changes in citrulline and lycopene in the watermelon fruit followed the same general pattern, being low in young fruit and increasing with fruit size, reaching peak levels at full maturity followed by a slight decrease in over-mature fruit.

INTRODUCTION

Watermelon is among the most valuable horticultural crops in the world and is also a healthy fruit due to its high lycopene content. Lycopene is a type of carotenoid that is also found in ripe tomato and pink grape fruit, and gives them a characteristic red color. Lycopene makes fundamental contributions to human health; lycopene intake has been particularly associated with protection from prostate cancer as well as a lowered risk of coronary heart disease and lung cancer (Le et al., 2005). The lycopene content in commercial watermelon has been reported to be 45.1–53.2 mg kg^{-1} fresh weight (fwt) (Heinonen et al., 1989; Tee and Lim, 1991; Mangels et al., 1993). These values are 60% higher than those reported for red tomato (mean value of 30.2 mg kg^{-1} fwt) (USDA-NCC, 2001). Current research indicates that lycopene concentration in red-fleshed watermelon can be as low as 12 mg kg^{-1} in some seeded cultivars and as high 100 mg kg^{-1} in some seedless cultivars. Triploid seedless watermelon varieties tended to have higher amounts of lycopene (>50.0 mg kg^{-1} fwt) than diploid seeded types and modern hybrids tend to be higher than heirloom cultivars (Perkins-Veazie et al., 2001; Perkins-Veazie and Collins, 2006).

Citrulline is a non-essential amino acid first identified from the juice of watermelon. Citrulline in wild watermelon contributes to oxidative stress tolerance under drought conditions as a novel hydroxyl radical scavenger (Akashi et al., 2001). Citrulline is used in the nitric oxide system in humans and has antioxidant and vasodilatation roles. Dietary supplements containing citrulline have been used to improve sexual stamina and erectile function. A method using gas chromatography–mass spectrometry (GC–MS) was developed to separate citrulline from glutamic acid, which co-elute when analyzed by high performance liquid chromatography. Watermelon is the richest known source of citrulline. Watermelons were analyzed by GC–MS to determine the citrulline content among varieties, types, flesh colors, and tissues. Citrulline content ranged from 3.9 to 28.5 g kg^{-1} dry weight (dwt) and was similar between seeded and seedless types (16.6 and 20.3 g kg^{-1} dwt, respectively). Red flesh watermelons had slightly less citrulline than the yellow or orange flesh watermelons (7.4, 28.5 and 14.2 g kg^{-1} dwt, respectively). The rind contained more citrulline than flesh on a dry weight basis (24.7 and 16.7 g kg^{-1} dwt, respectively) but a slightly less on a fresh weight basis (1.3 and 1.9 g kg^{-1} fwt, respectively) (Rimando and Perkins-Veazie, 2005).

Most eukaryotic genomes have numerous duplicated genes, many of which appear to have arisen from one or more cycles of polyploidy (genome doubling), either by allopolyploidy or autopolyploidy. The merging and doubling of two genomes sets in motion extensive modifications of the genome and/or transcriptome, creating cascades of novel expression patterns, regulatory interactions and new phenotypic variation for evaluation by natural selection. With doubled chromosomes, polyploidy plants may contain new agricultural characteristics which did not exist in their diploid progenitor; traits such as new resistance, increased metabolite production or new secondary metabolites have resulted from polyploidization (Soltis and Soltis, 2000). Many phenotypes of autotetraploid watermelon are changed compared to the diploid progenitor, such as the increased leaf guard cell and epidermal cell size, pollination and pollen germination are reduced, chlorophyll a, chlorophyll b and carotenoid content may increase and salt tolerance and disease resistance may increase (Liu et al., 2003a, b, c, 2005). The vitamin C and sugar content of autotriploid and autotetraploid in 9 watermelon varieties were shown to be higher than the diploid (Cheng et al., 2008).

The specific objective of this study was to determine changes in lycopene and citrulline contents in the fruit after diploid watermelons were artificially induced into autotetraploid and autotriploid. Changes in lycopene and citrulline development were also monitored as fruit developed. Results of this study will provide biological basis for watermelon fruit harvest and breeding of functional watermelon varieties.

MATERIALS AND METHODS

Plant Materials

Watermelons used for this study were grown in greenhouse of research station of Zhengzhou Fruit Research Institute, CAAS in spring 2009. Total 27 varieties were used for this experiment, and included 9 diploid inbred lines, their induced autotetraploid (9) and autotriploid (9). Hand pollination was done at flowering time and pollinated flowers were marked. The samples of different polyploidy of 7 red flesh varieties, including Fan Zu NO.2, Xin Qing , Mi Mei , Jing Mu , 02B4, Zheng Zhou NO.3 and 89 Xuan 9, were evaluated for determination lycopene and citrulline contents of ripe fruits. One yellow flesh variety (Huang Mei) was added for measuring citrulline content of mature fruits. The variety XYXA, along with its autotetraploid and autotriploid, was used to explore the changes of lycopene and citrulline contents during the development of fruit. Fruits were harvested at 15, 20, 25, 28, 31, 34, 37, and 40 days after pollination (DAP). All sampling was done by harvesting approximately 5g of tissue from the center of the watermelon heart, beat to serum with strip engine for measurement and stored at -20°C until assayed.

Statistical comparison of lycopene and citrulline for different polyploidy levels was done using SAS V 8.0 at $P<0.05$.

Determination of Lycopene

Frozen watermelon tissue (2–3 g/sample) was thawed and pureed using an electric glass homogenizer (DY89-2, Ningbo Scientz Biotechnology Co., Ltd, China) to break cell walls. The sample was diluted by adding 5 ml absolute ethyl alcohol. The diluted sample was then added to 20 ml absolute ethyl alcohol, 30 ml methanol and 80 ml 2% dichloromethane + petroleum ether mixture to extract lycopene. One extraction was sufficient to completely remove the color. The extracted solution was finally added to 100 ml and measured on a UV spectrophotometer (Beijing LabTech Inc. China) at 502 nm. The lycopene content was estimated from the absorbance readings calculation according to the specification curve in reference. Data was the mean of three replications. Lycopene content was estimated using the equation: (mg kg^{-1} FW) = A/0.3078/W×, where A = light absorption value on 502 nm, 0.3078 = slope of specification curve, W = the weight of sample (g), and F = dilution rate.

Determination of Citrulline

Three grams frozen watermelon flesh tissue were thawed and extracted with 15 ml of methanol:6M HCl (9:1) at 55°C in water bath for 20 min. The extract solution was decolorized with 10 g activated carbon, and then 1 ml of the filtered solution was diluted with 7 ml distilled water. One ml of the diluted solution was added to 4 ml distilled water, 2 ml of sulphuric acid:phosphoric acid (3:1 in volume), 0.25 ml 30 g/L diacetyl monoxime in order, and the solution was shaken in the dark in a closed hood. The solution was then heated in a 100°C water bath for 30 min and allowed to cool to room temperature. The final sample was measured with UV spectrophotometer at 490 nm. The citrulline content was calculated according to a calibration curve of an external standard citrulline. The data was the mean of three repeats. Citrulline content was estimated using the equation: (g kg^{-1} FW) = (0.1288A+0.0044)×F, where A = light absorption value on 490 nm, 0.1288 = slope of specification curve, 0.0044 = intercept of specification curve, and F = dilution rate.

RESULT AND DISCUSSION

Lycopene Content in Different Ploidy Watermelon

Lycopene contents were different between red flesh watermelon varieties and also varied with different ploidy levels (Table 1). The range of lycopene content among diploid watermelon fruit was from 33.2 to 54.8 mg kg^{-1} with a mean of 44.1mg kg^{-1}. The range of lycopene content among triploid was from 41.2 to 61.8 mg kg^{-1} and a mean of 49.8 mg kg^{-1}. The range of lycopene content among tetraploid was from 38.1 to 59.8 mg kg^{-1} and a mean of 50.9 mg kg^{-1}. The lycopene content of the triploid was higher than the tetraploid for all varieties except Fan Zu No. 2. The lycopene content in the triploid and tetraploid were higher than that of diploid with the exception of Fan Zu No. 2. Ploidy did not affect lycopene content in variety Fan Zu No. 2.

Lycopene Content Change during Fruit Development

Lycopene contents of variety XYXA fruits with different ploidy were measured during their development. The lycopene content of tetraploid, triploid and diploid watermelon fruit 15 DAP were 5.3, 3.8 and 3.4 mg kg^{-1} FW, respectively. Lycopene contents increased with fruit ripening. The maximum value of the tetraploid and triploid was 62.3 mg kg^{-1} FW on 37 DAP and 55.6 mg kg^{-1} FW on 34 DAP. The maximum value of diploid was 42.7 mg kg^{-1} FW on 34 DAP, but the lycopene content was lower than polyploids. The lycopene content decreased slightly in over-mature fruit. On 40 DAP, the lycopene content of the tetraploid, triploid and diploid were 52.6, 42.5 and 40.4 mg kg^{-1} FW, respectively. As shown by the results of this study (Fig. 1), lycopene content was low in young fruit and increased rapidly with fruit ripening; the maximum value was found in mature fruit and decreased slightly in over-mature fruit. The time of maximum value in diploid was slightly earlier than triploid, and the triploid was slightly earlier than the tetraploid.

Citrulline Content in Different Ploidy Watermelon

The citrulline content was not as consistent, but it tended to vary in different ploidy levels of watermelon. The citrulline content in diploid watermelon ranged from 1.73 to 3.26 g $kg^{-1}$with average 2.27 g kg^{-1}. The citrulline content in triploid ranged from

2.01 to 3.46 g kg^{-1} with average 2.71 g kg^{-1}. The citrulline content in the tetraploid ranged from 2.01 to 3.07 g kg^{-1} with average 2.45 g kg^{-1}. The citrulline content of the triploid was tended to be higher than the tetraploid with 7 varieties evaluated, but the differences were not significant. The triploid and tetraploid tended to be higher than the diploid, but the differences were not significant (Table 2).

Citrulline Content Change during Fruit Development

Citrulline contents of watermelon variety XYXA with different polyploidy levels were measured during their fruit development. On 15 DAP, the citrulline content of the tetraploid, triploid and diploid were 0.76, 0.5 and 0.35 g kg^{-1} FW, respectively. A slow increase of citrulline in watermelon fruit was detected after the 15 DAP. Citrulline reached a maximum level of 1.61 g kg^{-1} FW in tetraploid fruit on 34 DAP. The maximum levels of citrulline were 1.20 g kg^{-1} FW on 34 DAP in triploid fruit and 1.02 g kg^{-1} FW on 31 DAP in diploid fruit. The citrulline content decreased slightly after full maturity. On 40 DAP, the content of the tetraploid, triploid and diploid were 1.13, 0.73 and 0.65 g kg^{-1} FW, respectively. The above results show that citrulline content of watermelon was low in young fruit, increased as the fruit matured, and reached maximum levels in mature fruit and decreased slightly in over-mature fruit. The occurrence of maximum value was earlier in diploid fruit and followed by the triploid and tetraploid fruit (Fig. 2).

Great genetic variation of lycopene content was observed in this study. The maximum value of lycopene content in red flesh was 54.8 mg kg^{-1}, and the minimum value was 33.2 mg kg^{-1} (a 65% variation). Similar genetic variation of citrulline content was observed, with a maximum value of 3.26 g kg^{-1}, and a minimum value of 1.73 g kg^{-1} (a 47% variation). This research confirmed that watermelon is good source of lycopene and citrulline. The variation demonstrated that breeding for higher levels of lycopene and citrulline is possible.

Bangalore et al. (2008) reported that lycopene extraction from overmature watermelon may possibly be boosted from the use of higher shear force and/or longer shearing treatment times during grinding/blending to breakdown asymmetrical structures and release more lycopene. In order to release the lycopene of flesh, we beat to serum with strip engine first, then pureed using electric glass homogenizer. So this is a better method for lycopene extraction as flesh cells were fully broken during the procedure.

As previously mentioned, many phenotypic changes occur with the doubling of watermelon chromosomes. It was previously known that watermelon is a natural rich source of lycopene and citrulline, but this is the first report of changes in lycopene and citrulline levels induced by artificial autopolyploidization. In this study 9 diploid inbreds were induced to autotetraploid and autotriploid. Lycopene and citrulline of all the materials were measured using mature fruit samples. The results showed that most polyploid watermelon fruit had more lycopene and tended to contain more citrulline than their diploid progenitor. These results indicate that chromosome doubling affects the expression of genes involved in the bio-synthesis of these secondary substances. This study is also the first report that lycopene and citrulline development in watermelon fruit shows a similar pattern; it is low in young fruit, increases with fruit size, reaches a maximum level in mature or near-mature fruit, and decreases slightly after maturity. This research provides a basis of proper fruit maturity and breeding for high levels of lycopene and citrulline in watermelon.

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